THE LARVAE OF MALAYAN ANOPHELES.

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Introductory.

It was long ago recognised by students of the biology of mosquitos that differences in certain larval characters could be employed for the separation of the Culicidae into groups and even for the recognition of individual species. Present-day knowledge of this subject in relation to the Anopheline group is largely based on the work of Grassi in Italy and James and Christophers in India.

It has been stated that in Anopheline larvae the characters are not sufficiently constant to be reliable for the identification of species. Recent studies have shown, however, that those differences which were formerly believed to be variations in the

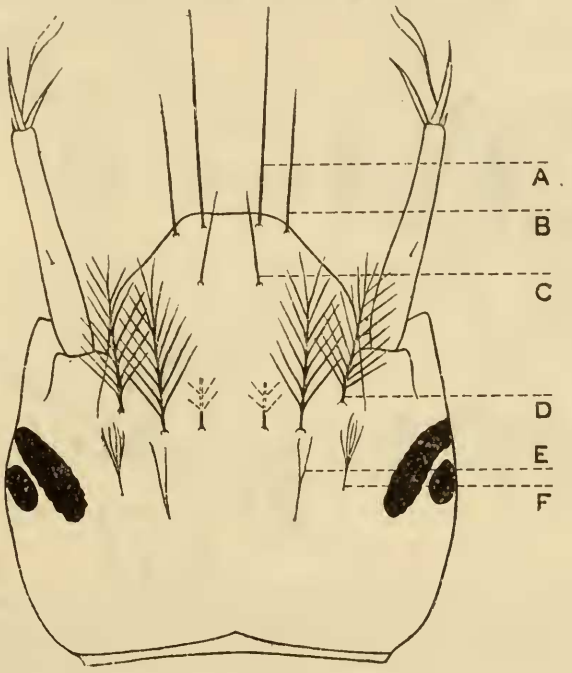

Fig. 1. Diagram to show position of hairs on dorsal surface of the head of an *Anopheles* larva (for explanation see text).

larva of a single species, are in reality changes of a constant kind associated with successive phases of its growth and that there is a high degree of constancy in the specific characters of larvae at identical stages of growth. In the examination of many thousands of Anopheline larvae taken in the Malay Peninsula and neighbouring islands, it has been possible to recognise with certainty the larvae of most of the species found in this region. A certain assemblage of characters is diagnostic of the species.

The characters which have been found useful for the identification of species are the form and relative position of certain appendages of the cuticle, hairs and similar structures, designed to subserve functions of sensation and the maintenance of

position at the water surface for breathing and feeding. The accompanying diagram (fig. 1) shows the position of these appendages on the upper or dorsal surface of the head in an *Anopheles*. A and B are the inner and outer pairs of *anterior clypeal hairs*, C the pair of *posterior clypeal hairs*; these hairs differ notably in form and position in different species and in the same species at different stages of its growth. D is one of a row of six *frontal* hairs between the bases of the antennae, E and F are the inner and outer pairs of *occipital* hairs; in a few species only are these groups of value for identification. There is also present in certain species a branched hair on the shaft of the antenna.

It is not only in *Anopheles* that the form and arrangement of the hairs on the dorsum of the larval head are characteristic; in other groups of CULICIDAE they are also of generic and specific value. In Malayan species of the Culicine genus

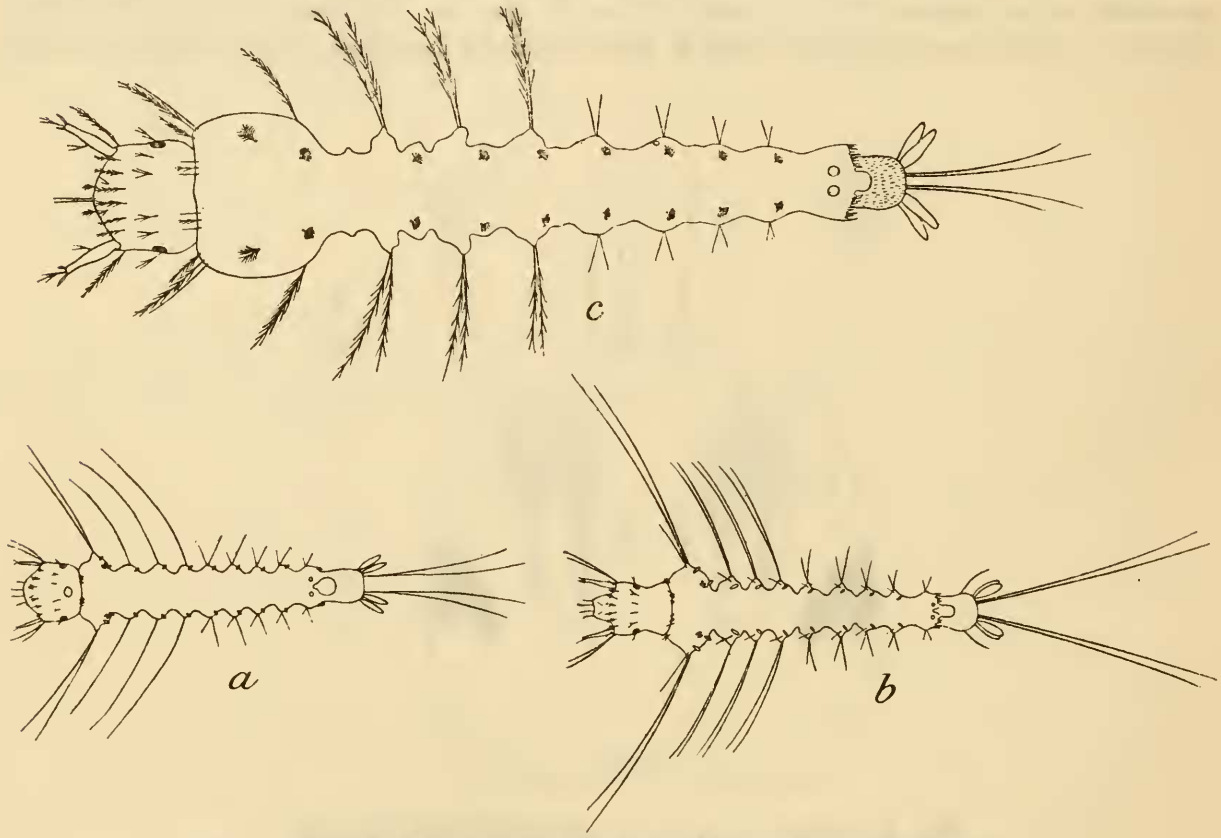

Fig. 2. Larva of *Anopheles sinensis*, Wied.; *a*, newly hatched; *b*, after first moult; *c*, after second moult.

Stegomyia and of the nearly related genera *Armigeres*, *Ochlerotatus* and *Danielsia*, the general arrangement of larval head hairs is the same, while their form differs in the different species. Larvae of the genus *Uranotaenia* are characterised by the presence of large spinous setae on the dorsum of the head.

Growth Changes in Anopheline Larvae.

During the growth of Anopheline larvae of all species notable changes take place in the form and arrangement of the clypeal hairs of the head and in the leaflets of the thorax and abdomen. Drawings of the larvae of *Anopheles sinensis*, Wied., at different

stages in its growth (fig. 2) show the details of these changes in that species. Thus in the early stages the anterior and posterior clypeal hairs are simple, in the later stages the outer anterior clypeal hair is thickly branched, the inner one being simple, and the posterior clypeal hair is slightly branched. It will be observed too that the relative positions of the anterior clypeal hairs also differ, the middle pair being much closer together in the later stages.

In the earlier stages of most Anopheline larvae leaflets varying in number and position are present on the dorsal surface of the thorax and segments of the abdomen. In later stages these are transformed, on the thorax some to plumose or branched hairs and some to stellate tufts, on the abdomen to stellate tufts only. The form of the leaflets in the stellate tufts of mature larvae is of diagnostic value, as is also the number of such stellate tufts borne by the larva. Two groups of three hairs situated on the front edge of the thorax, the sub-median anterior thoracic hairs (fig. 3, *b*, A), are also of value in the separation of certain species. In the early stages of Anopheline larvae these hairs are represented by leaflets, their form in the later stages of *A. barbirostris* and *A. sinensis* is shown in fig. 3.

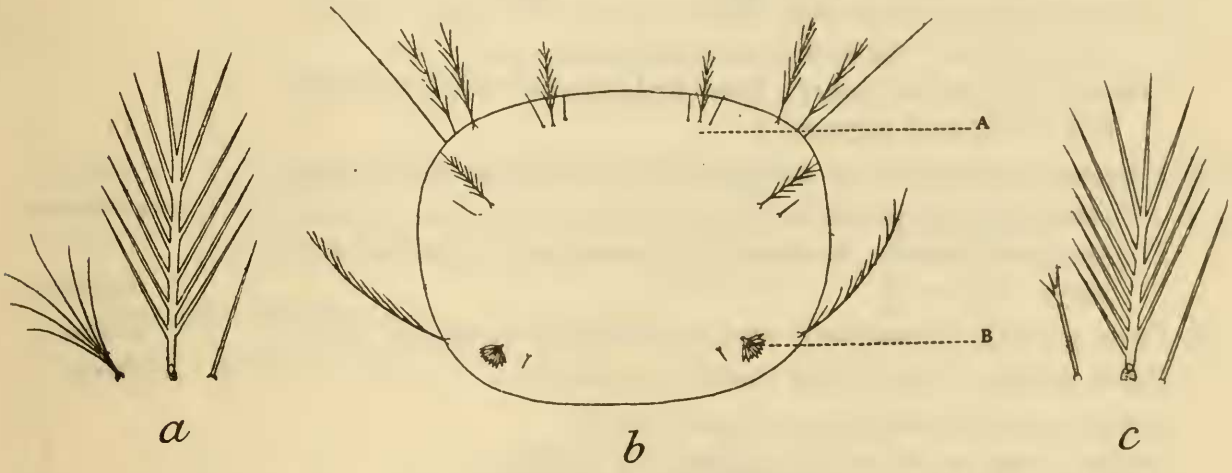

Fig. 3. Thoracic hairs of Anopheline larvae; *a*, sub-median anterior thoracic hairs of *A. barbirostris*; *b*, diagram to show position of hairs on the thorax of an *Anopheles* larva; (A) sub-median anterior hairs; *c*, sub-median anterior thoracic hairs of *A. sinensis*.

In previous papers (1912, 1913, 1914) I have described the mature larvae of a number of Malayan Anophelines and C. Strickland (1914) has described others. In the present paper it is proposed to describe and illustrate the salient features of the mature forms of known larvae and to indicate by means of a synoptic table a method of identification of species. As all the larval structures here referred to are paired, description of them is made easier by reference to those of one side only.

Larvae may conveniently be examined in a drop of water on a glass slide, the larva having been immobilised by exposing it to chloroform vapour or killed by adding a drop of weak formalin solution. They may be mounted as permanent specimens in weak formalin solution (5 per cent.) in a cell formed of a ring of paraffin.

Key to the known mature larvae of Malayan Anophelines.

1. Shaft of antenna with a branched hair 2
   Shaft of antenna without a branched hair 5
2. Outer anterior clypeal hair thickly branched; abdominal segments with large stellate tufts 3
   Outer anterior clypeal hair with few branches; abdominal segments without stellate tufts .. .. 13. *umbrosus.*
   Outer anterior clypeal hair simple or bifid terminally 4
3. Innermost sub-median anterior thoracic hair (fig. 3) branched from base .. .. 4. *barbirostris.*
   Innermost sub-median anterior thoracic hair with simple stem and four or five short branches terminally .. .. 11. *sinensis.*
4. Posterior clypeal hair short, branched .. .. 2. *aitkeni.*
   Posterior clypeal hair long, simple (C. Strickland) .. .. 3. *asiaticus.*
5. Inner and outer anterior clypeal hairs branched 6
   Inner and outer anterior clypeal hairs simple, or inner only with minute lateral branches 9
6. Posterior clypeal hairs long, simple; no stellate tufts on thorax or abdominal segment i 7
   Posterior clypeal hairs short, branched; stellate tufts on thorax and abdominal segment i 8
7. Filaments of leaflets of stellate tufts on mid-abdominal segments sharply pointed .. .. 9. *maculatus.*
   Filaments of leaflets of stellate tufts on mid-abdominal segments truncated .. .. 6. *karwari.*
8. Outer anterior clypeal hair with short lateral branches .. 1. *aconitus.*
   Outer anterior clypeal hair thickly branched .. .. .. 5. *fuliginosus.*
9. Stellate tufts on abdominal segments i–vii 10
   Stellate tufts on abdominal segments ii–vii 11
   Stellate tufts on abdominal segments iii–vii 12
10. Posterior clypeal hairs closer together between inner anterior clypeal hairs .. .. 10. *rossi* var. *indefinitus.*
11. Stellate tuft on thorax fully developed .. .. 7. *kochi.*
    No stellate tuft on thorax .. .. 8. *leucosphyrus.*
12. Outer anterior clypeal and posterior clypeal hairs very short 12. *tessellatus.*

1. **Anopheles aconitus,** Dönitz, (=albirostris, Theo.), (fig. 4).

Average length at maturity 3·5 m.m.

Head: *Anterior clypeal hairs.* Inner hair long and stout with numerous short lateral branches. Outer hair about half the length of the inner and similarly branched. *Posterior clypeal hairs* short, branched from the base into four or five divisions. *Occipital hairs* short, branched from the base into four or five divisions.

Thorax: On the posterior quadrant a fully developed stellate tuft composed of twelve to fourteen long narrow leaflets without terminal filaments.

Abdomen : On segment i. a fully developed stellate tuft composed of ten to twelve short narrow leaflets with ill-defined terminal filaments. On segments ii.-vii. large stellate tufts composed of fifteen to eighteen leaflets with well defined terminal

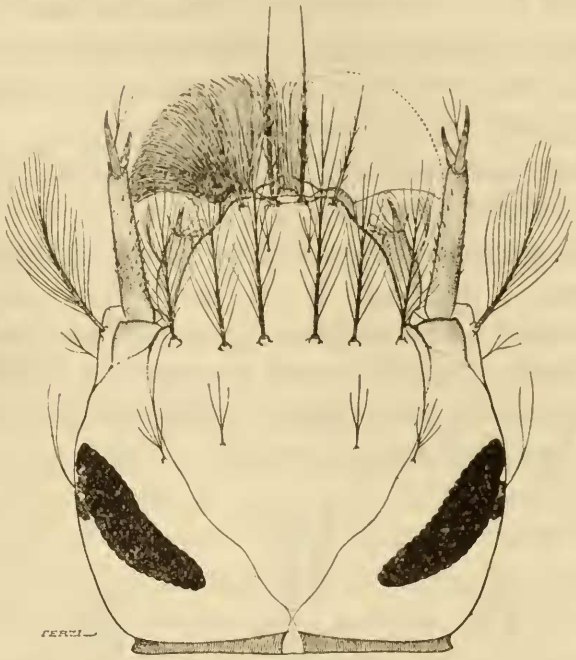

Fig. 4. *Anopheles aconitus*, Dön.

filaments. The average total length of the leaflets on the mid-abdominal segments is 0·08 mm. ; the average relation of length of filament to total length of leaflet is as 1·5 to 4. The filament is deeply indented at its base and is sharply pointed.

2. **Anopheles aitkeni,** James, (figs. 5, 6).

Average length at maturity 3·5 mm.

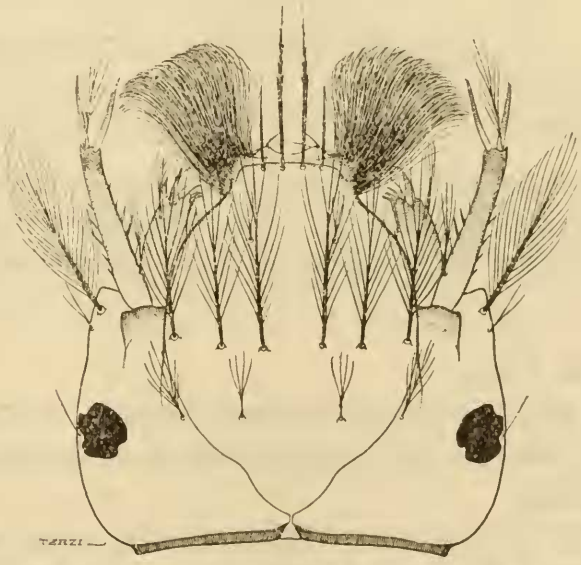

Fig. 5. *Anopheles aitkeni*, James (Type I.).

Head : *Anterior clypeal hairs.* Type I. (fig. 5) : Inner hair long and stout, middle third with short lateral branches, elsewhere the shaft is bare ; outer hair short, one-

third the length of the inner, simple. Type II. (fig. 6): Inner hair long, basal third or stem stout and bare; at the end of the stem the hair branches into three to six divisions, each equal to twice the length of the stem; outer hair short, usually simple, but may be bifid at its extremity. *Posterior clypeal hairs* short, branched from the base into three or four divisions. *Occipital hairs* short, branched, the inner with three divisions, the outer with five or six divisions. *Antenna* carrying a stout branched hair placed dorsally on the shaft near the base.

Thorax: On the posterior quadrant a fully developed stellate tuft composed of long narrow leaflets without filaments.

Abdomen: Segment i. carries a stellate tuft composed of five to seven short narrow leaflets without filaments. Segments ii.–vii. carry fully developed stellate tufts composed of fifteen to twenty broad leaflets with well defined filaments. The average length of the leaflet on the mid-abdominal segments is 0·075 mm. The average relation of length of filament to total length of leaflet is as 1 to 4. The filament is deeply indented at its base and sharply pointed.

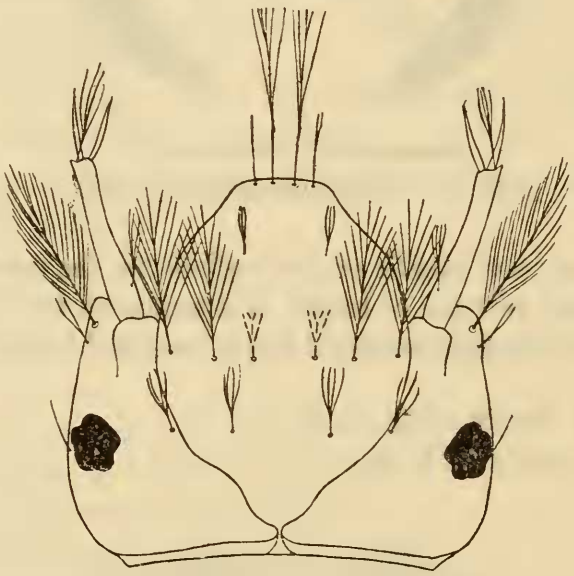

Fig. 6. *Anopheles aitkeni*, James (Type II.).

Alone among the larvae of Malayan Anophelines that of *aitkeni* exhibits variation in the form of the anterior clypeal hairs. Two principal types are here described, but it has been observed that combinations of these occur, even on different sides of the same larva.

3. **Anopheles asiaticus,** Leic.

The mature larva of this rare species was first described by C. Strickland (Parasitology, vii, p. 12, 1914), who has kindly permitted me to examine his specimens and to reproduce his description.

Head: Clypeal hairs. (i) Anterior: The internal hairs are long, stout, and close together, as in members of the *Myzorhynchus* group, and are bifurcate about their middle. (ii) The *external* hairs are about half the length of the internal, stout, projecting forwards and outwards, and simple. (iii) *Posterior*: These hairs are a little longer than the external ones and are simple and delicate.

The *frontal hairs* are six in number as usual, but only the two external ones are branched. The four internal hairs are very delicate. The *antennae* each possess a branched hair, as in *barbirostris* and *sinensis*, and the Indian species *lindesayi*, but it is situated on the *external* side of the antenna.

Thorax : There are no palmate hairs on the thorax. There is a deposit of black pigment in the form of a Maltese Cross, the posterior limb of which is bilobed.

Abdomen : In this region of the body the palmate hairs are slightly developed on the 2nd segment, well developed from the 3rd to 7th segment ; they are large and pigmented. Each leaflet is long and rather slender, terminating in a long filament without any very jagged shoulder. There is a dense black spot on the dorsum of the 4th segment."

A hair situated on the dorsum of the head near the base of the antenna (" basal hair " of James and Liston) is of unusual form in this species. In most Anophelines this hair is stout and feathered—in *asiaticus* it is long, slender and bifurcate terminally. The occipital hairs are short, slender and simple. The average length of the leaflet in the stellate tufts of the mid-abdominal segments is 0·08 mm. The average relation of length of filament to total length of leaflet is as 1 to 2·5.

I have found in the water of cut bamboos an immature Anopheline larva which is perhaps one of the early stages of *asiaticus*. It differs from the mature form described by Strickland in the form of certain of the head hairs. In my specimen the inner anterior clypeal hair has four to five lateral branches, the " basal hair " consists of a simple stem with a swollen extremity carrying a tuft of hairs (as in the larva of the Indian species *culiciformis*). The posterior clypeal hairs are minute, only about half the length of the outer anterior clypeal hairs. The outer pair of frontal hairs are branched as in *asiaticus*, the median pair short and simple, the inner pair long and bifurcate. A branched hair is borne on the antenna and stellate tufts are present on abdominal segments iii. to vii. only. There is a deposit of pigment of irregular form in the cuticle of the dorsum of the thorax.

4. **Anopheles barbirostris,** Van der Wulp, (fig. 7).

Average length at maturity 5·5 mm. The larva of this species is usually dark-coloured, with light bands across the front of the thorax and abdominal segments iii., v. and viii.

Head : *Anterior clypeal hairs.* Inner hairs close together, long and simple ; outer hairs shorter, thickly branched. *Posterior clypeal hairs* short, branched, with three or four divisions. *Occipital hairs* short, both branched, with seven or eight divisions. *Antenna.* On the inner surface about the middle the shaft carries a long branched hair.

Thorax : Sub-median anterior thoracic hairs (fig. 3) as follows, from the mid line :—(1) a short hair branched near the base, with six divisions ; (2) a long stout plumose hair ; and (3) a long simple hair externally. On the posterior quadrant a fully developed stellate tuft composed of twelve to fifteen long narrow leaflets without filaments (fig. 3, *b*, B).

Abdomen : Segment i. carries a fully developed stellate tuft composed of eight to ten long narrow leaflets without filaments. Segments ii.–vii. carry fully developed stellate tufts composed of about twenty long broad leaflets. The average length

of the leaflets on the mid-abdominal segments is 0·11 mm. The average relation of length of filament to total length of leaflet is as 1 to 2·2. The filament is marked off from the remainder of the leaflet only by minute indentations along its edges.

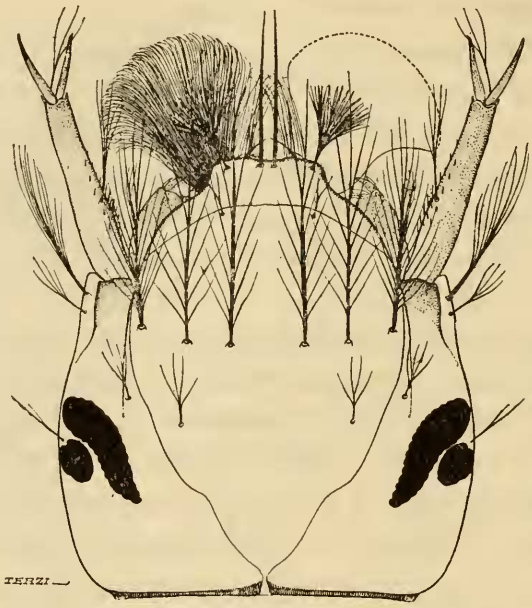

Fig. 7. *Anopheles barbirostris*, Wulp.

5. **Anopheles fuliginosus,** Giles, (figs. 8, 9).

Average length at maturity 4·0 mm.

Head: *Anterior clypeal hairs.* Inner hair long and stout, with short lateral branches; outer hair about half the length of the inner, thickly branched. *Posterior*

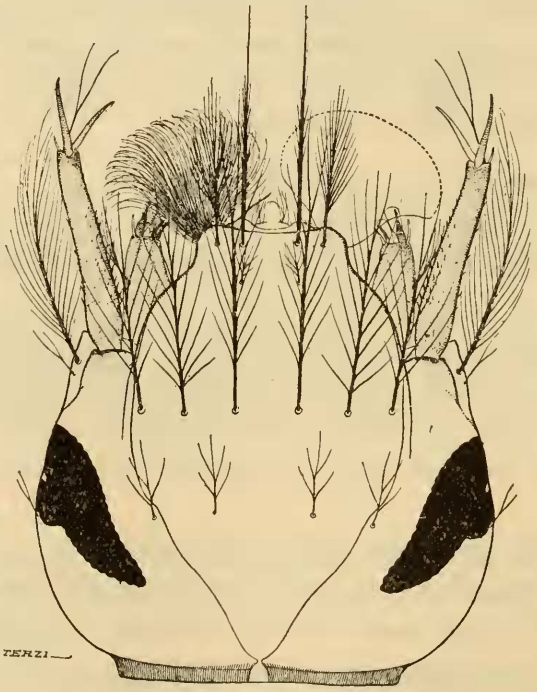

Fig. 8. *Anopheles fuliginosus*, Giles

clypeal hairs short, branched from the base, with five or six divisions. *Occipital* hairs, both short, branched, with five to seven divisions.

THORAX: On the posterior quadrant a fully developed stellate tuft, composed of twelve to fifteen long narrow leaflets without filaments.

ABDOMEN: Segment i. carries a fully developed stellate tuft composed of eight to ten long narrow leaflets with ill-defined filaments. Segments ii.–vii. carry large stellate tufts composed of fifteen to twenty broad leaflets with well defined filaments (fig. 9). The average length of the leaflets on the mid-abdominal segments is 0·085 mm. The average relation of length of filament to total length of leaflet is as 1 to 2·5. The filament is deeply indented at its base and is sharply pointed.

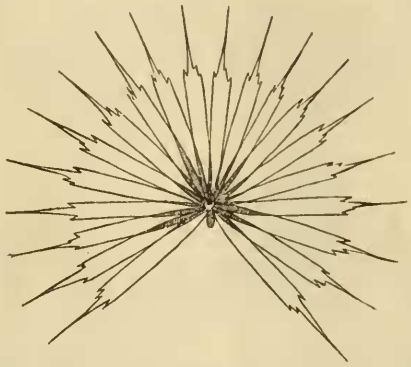

Fig. 9. *Anopheles fuliginosus*, Giles; stellate tuft.

6. **Anopheles karwari,** James, (fig. 10).

Average length at maturity 4·5 mm.

HEAD: *Anterior clypeal hairs.* Inner hair long and stout, with fine lateral branches; outer hair shorter and similarly branched. *Posterior clypeal hairs* long, slender, sometimes with lateral branches. *Occipital hairs.* Inner long, simple; outer long, branched, with four or five divisions.

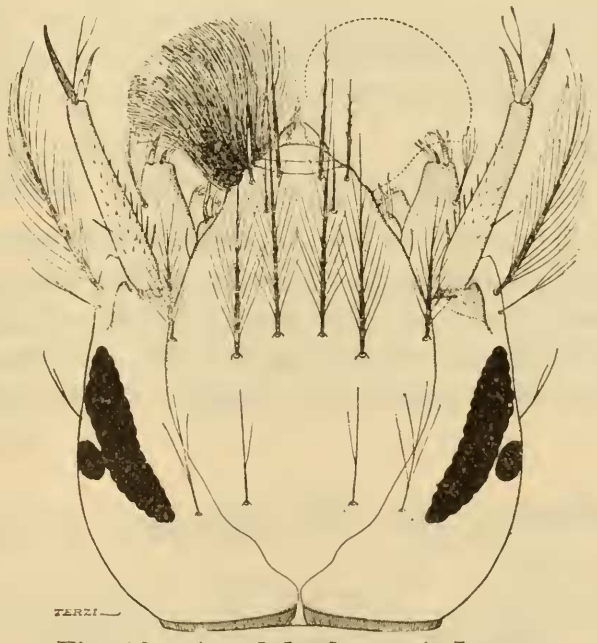

Fig. 10. *Anopheles karwari*, James.

THORAX without a stellate tuft.

ABDOMEN: Segment ii. carries a stellate tuft composed of eight to ten narrow leaflets without filaments. Segments iii.–vii. carry stellate tufts composed of fifteen

to eighteen short, broad leaflets with filaments. The average length of the leaflets on the mid-abdominal segments is 0·06 mm. The average relation of length of filament to total length of leaflet is as 1 to 6. The short filament is indented at its base and truncate.

7. **Anopheles kochi,** Dönitz,* (fig. 11).

Average length at maturity 4·0 mm.

Head: *Anterior clypeal hairs.* Inner hair long and slender, generally with few lateral branches; outer hair very short, about one-fourth the length of the inner, simple. *Posterior clypeal hairs,* short, simple. *Occipital hairs,* both long, inner simple, outer branched.

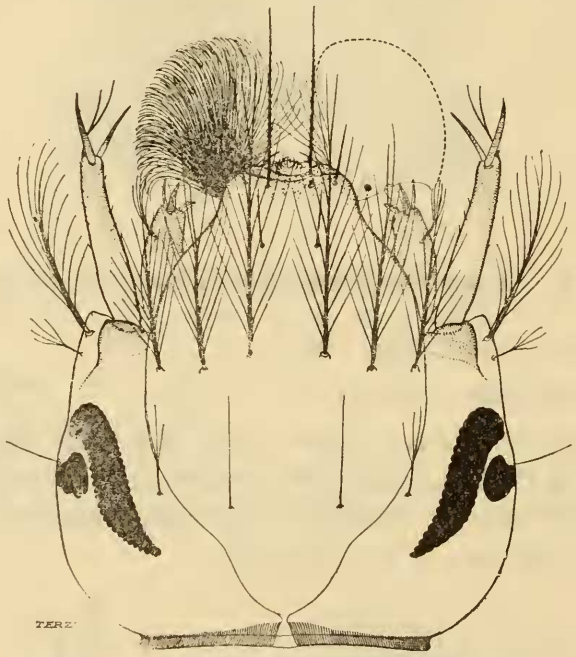

Fig. 11. *Anopheles kochi,* Dön.

Thorax: On posterior quadrant a fully developed stellate tuft composed of ten to twelve long narrow leaflets without filaments.

Abdomen: Segment ii. carries a stellate tuft composed of five to seven short narrow leaflets without filaments. Segments iii.–vii. carry stellate tufts of fifteen to eighteen short narrow leaflets with filaments. The average length of the leaflets on the mid-abdominal segments is 0·60 mm. The average relation of filament to total length of leaflet is as 1 to 3·5. The filament is slightly indented at its base and tapers gradually to a point.

8. **Anopheles leucosphyrus,** Dönitz, (fig. 12).

Average length at maturity 5·0 mm.

Head: *Anterior clypeal hairs.* Inner hair very long and slender, simple; outer hair about one-half the length of the inner, simple. *Posterior clypeal hairs,* long, simple. *Occipital hairs,* both long, inner simple, outer branched.

Thorax without a stellate tuft.

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* See also Bull. Ent. Res., v., p. 129 (1914).

Abdomen: Segments ii. and vii. carry fully developed stellate tufts composed of eight to twelve long narrow leaflets without filaments. On segments iii. to vi. the leaflets number fifteen to eighteen and have well defined filaments. The average

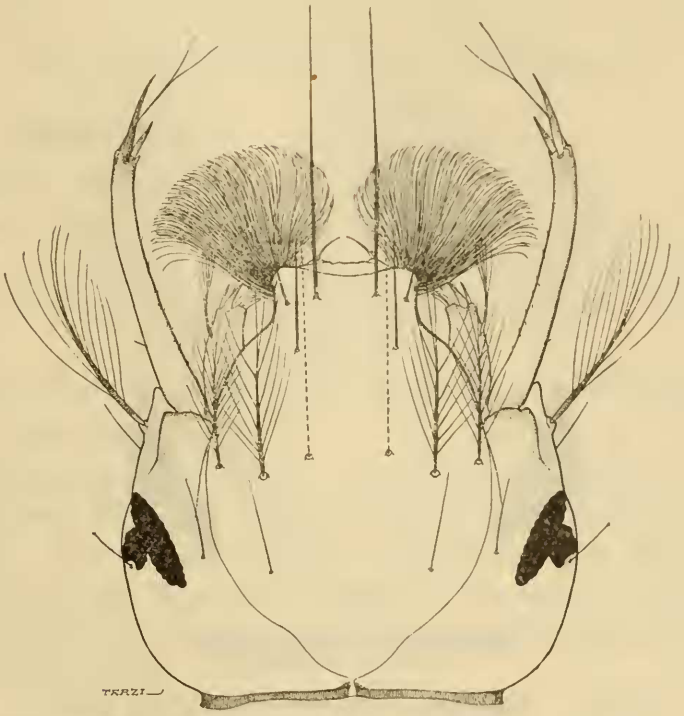

Fig. 12. *Anopheles leucosphyrus*, Dön.

length of the leaflets on the mid-abdominal segments is 0·10 mm. The average relation of length of filament to total length of leaflet is as 1 to 2·5. There are several indentations at the base of the filament, which is sharply pointed.

9. **Anopheles maculatus,** Theo. (*cf.* fig. 10).

Average length at maturity 4·5 mm.

The larva of this species differs from that of *karwari* only in the form of the leaflets of the stellate tufts of the mid-abdominal segments. In *maculatus* the average length of these leaflets is 0·06 mm. The average relation of the length of the filament to the total length of the leaflet is as 1 to 4. The filament is deeply indented at the base and is sharply pointed.

10. **Anopheles rossi,** var. **indefinitus,** Ludl. (figs. 13, 14).

Average length at maturity 5·0 mm.

Head: *Anterior clypeal hairs.* Inner hair long, slender, simple; outer hair, short, simple. *Posterior clypeal hairs* short, simple, being placed unusually far forwards on the clypeus and between the inner pair of anterior clypeal hairs. This arrangement of clypeal hairs is characteristic for this variety. *Occipital hairs.* Inner hair long, simple; outer hair branched.

Thorax without a stellate tuft.

Abdomen: Segment i. carries a stellate tuft composed of five to seven long narrow leaflets without filaments. Segments ii.-vii. carry stellate tufts composed of fifteen to eighteen long narrow leaflets with well defined filaments (fig. 14). The average

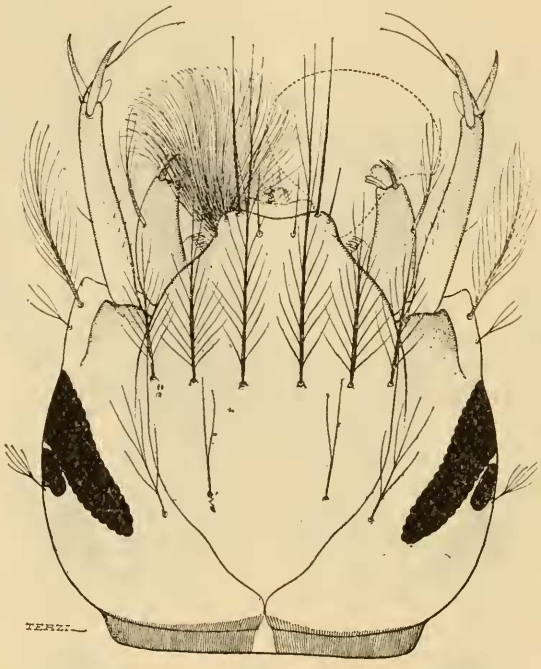

Fig. 13. *Anopheles rossi* var. *indefinitus*, Ludl.

length of the leaflets on the mid-abdominal segments is 0·08 mm. The average relation of length of filament to total length of leaflet is as 1 to 2. The filament is deeply indented at its base and is unusually long and slender.

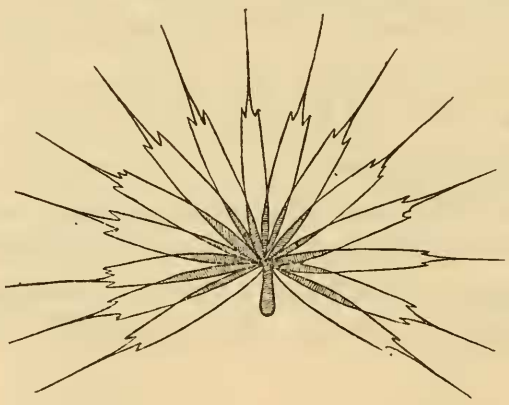

Fig. 14. Stellate tuft of *A. rossi* var. *indefinitus*, Ludl.

11. **Anopheles sinensis,** Wied. (*cf.* fig. 7).

Average length at maturity 5·5 mm.

The larva of this species can usually be differentiated by the naked eye appearances from that of *barbirostris*. The larva of *sinensis* is coloured greenish or yellowish brown, instead of black, as is *barbirostris*; also *sinensis* lacks the whitish bands which are so characteristic of *barbirostris*.

The only microscopic character by which these larvae may be differentiated is the form of the innermost of the submedian anterior thoracic hairs (fig. 3). In *sinensis* this hair has a long simple stem, which is seen when highly magnified to be branched near the end. This minute difference has proved to be constant in a large number of specimens of cast larval skins which have been examined. Other larval characters in which it has been alleged that differences exist between *sinensis* and *barbirostris* have been found to be identical in the two species.

12. **Anopheles tessellatus,** Theo.*

Average length at maturity 4·0 mm.

Head: *Anterior clypeal hair.* Inner hair stout, of medium length, with minute lateral branches; outer hair short, only about one-fifth the length of the inner, simple. *Posterior clypeal hairs* short, slender and simple. *Occipital hairs* short, both branched.

Thorax: On the posterior quadrant a rudimentary stellate tuft composed of five to seven narrow lanceolate leaflets.

Abdomen: Segments iii.–vi. carry fully developed stellate tufts composed of leaflets with ill-defined filaments. On segment vii. the leaflets are narrowly lanceolate, without filaments. The average length of the leaflets on the mid-abdominal segments is 0·06 mm. The average relation of length of filament to total length of leaflet is as 1 to 3. The indentations at the base of the filament are minute and the filament tapers gradually to a point.

13. **Anopheles umbrosus,** Theo. (fig. 15).

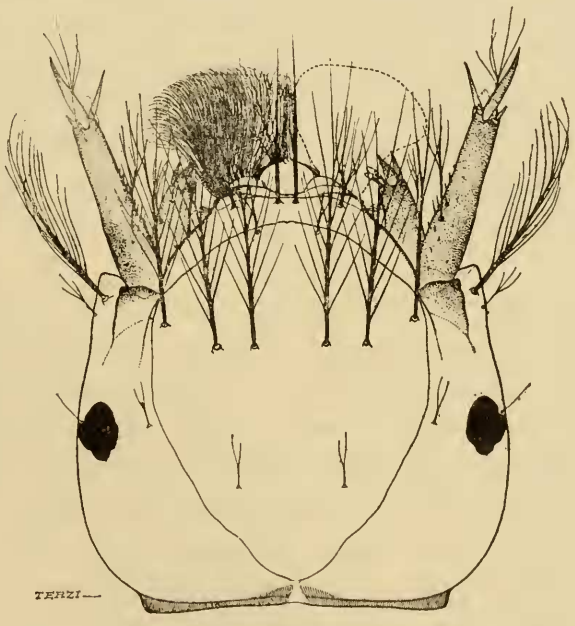

Fig. 15. *Anopheles umbrosus,* Theo.

Average length at maturity 5·0 mm.

Head: *Anterior clypeal hairs.* Inner hair long, simple; outer hair with few branches as compared with the allied species *sinensis* and *barbirostris*; the usual

* See also Bull. Ent. Res., iv., p. 129 (1913).

arrangement is division of the stem near the base into two, each of which bears two or three branches. *Posterior clypeal hairs* short, branched, with three or four divisions. *Occipital hairs* stout, branched. *Antenna* carrying on its inner side a long stout branched hair.

Thorax: Sub-median anterior thoracic hairs as in *sinensis*. No stellate tuft.

Abdomen: There is no stellate tuft on any segment of the abdomen. This is a unique character; all other Anopheline larvae so far described bear stellate tufts on several segments of the abdomen. In this species the corresponding structure is a much-branched plumose hair.